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Stimulation of storage tissues of higher plants by zinc sulphate

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The fact that chemical poisons under certain conditions and in certain quantities are of a stimulating nature to plants has been well established through the work of Raulin, Richards, Ono, Yashudi, Latham, Watterson, and others. A complete list of the references on the work done in this field may be found in the papers by M. E. Latham * and A. Watterson.† These investigations, however, have been conducted chiefly upon lower plants and the seedlings of higher ones. Little has been done upon the mature forms of the higher plants, or upon their various parts and tissues. This article attempts to describe some investigations on the effect of zinc sulphate stimulation on the storage tissues of some of the higher plants.

The work was done in the botanical laboratory of Barnard College, Columbia University, during the years 1907 and 1908, under the direction of Dr. H. M. Richards, to whom the writer is deeply indebted for his kind interest and assistance.

The investigation was in two parts, — first, the effect of solutions of zinc sulphate of various strengths upon the formation of periderm and callus, and second, the effect of such solutions upon the respiration of the tissue.

All zinc sulphate solutions weaker than normal were made up

* Latham, M. E. Stimulation of *Sterigmatocystis* by chloroform. Bull. Torrey Club **32**: 337. 1905.

† Watterson, A. The effect of chemical irritation on the respiration of fungi. Bull. Torrey Club **31**: 291. 1904.

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from a normal solution, prepared as follows: The zinc sulphate used was Merck's "highest purity reagent." For 100 c.c. of a normal solution, 14.3786 gm. of $ZnSo_4$ would be theoretically required (allowing $7H_2O$ for water of crystallization). 14.51923 gm. were weighed out, this differing from the required by 0.14063 gm. 14.3786 : 100 :: 0.14063 : x. $X = 0.978$ c.c. of water. Therefore, 100.978 c.c. of water added to 14.51923 gm. of zinc sulphate makes a normal solution. The water was the distilled water used in the laboratory. This was used also for washing all apparatus.

The apparatus used, and the results obtained in regard to the first part of the work, that is, the effect upon the periderm and callus formation, will first be described.

The glass dishes into which the specimens were to be placed, after being washed in distilled water, were put in the steam sterilizer, and heated at boiling point for not less than thirty minutes. The first experiments were made in an atmosphere dried by having a solution of potassium hydrate present in a small open dish. But the specimens dried out too much and were unsatisfactory for examination. Thereafter the experiments were conducted in a saturated atmosphere, the dishes being prepared in the following manner. All glassware was washed as stated above before using. The bottom of a glass dish was covered about a half or three quarters of an inch deep with very moist sphagnum. Then a side of a Petri plate which fitted closely into the glass dish was placed on the sphagnum. Then another glass dish slightly larger than the first was placed over it, forming a closely fitting cover. After heating in the steam sterilizer as aforesaid and cooling, the cover was lifted as little as possible at the side, and the specimens placed in an upright position on the Petri plate (see FIGURE 1, *a*). The covers of the dishes were never taken completely off during the experiments. They were always plainly labeled with the strength of the solution into which the specimens had been dipped, and the date the experiment was started, so no errors could arise through confusing the dishes—for example, "N/12 $ZnSO_4$, Jan. 23/09." The dishes for the control and the poisoned specimens were always prepared at the same time and in exactly the same manner. In some cases where it was thought possible that a variation in results was due to a difference in the atmosphere in the two dishes, the

experiment was repeated, placing both the control and the poisoned specimens in the same dish.

For cutting the tissues a copper tube was first used. Then a silver tube was substituted, as it was thought that the copper might have some effect upon the tissue with which it came in contact. But on comparing results, it appeared that the copper had had no effect upon the tissue. The tube, and the scalpel and forceps used in handling the specimens, were always placed in boiling water for a few minutes before each time they were used. The poisoned specimens and the control specimens were always taken from the same plant and were prepared in the following way: The potato, kohl-rabi, or whatever plant was used, was thoroughly washed and dried. Then the silver tube was pushed through the fleshy part of the plant root or tuber, and the cylinder thus cut was pushed out onto a piece of clean filter-paper. It was then usually cut into pieces 20 mm. long,—the tube was 7 mm. in diameter. The part closer than 3 or 4 mm. to the end was never used. In the cases of the roots, where there was a longitudinal axis, the pieces were cut with the longitudinal axis. This was repeated until a sufficient number of pieces of each plant was cut.

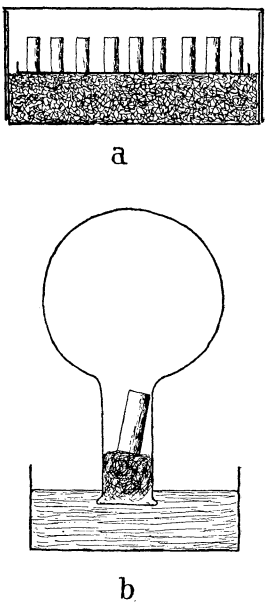

FIGURE 1. *a*, Diagram showing the manner in which the cylinders of tissue were placed in the culture dishes; *b*, diagram showing arrangement for collecting CO_2 given off for relatively short periods from small portions of tissue. The liquid in the dish is mercury.

Three pieces of each kind successively were put into a small bottle containing the zinc sulphate solution of required strength; and the same number was put into a like bottle containing distilled water. This was done in order that all factors of moisture, etc.—except that of the poison—might be as nearly the same as possible. After being left in the solution, or the water, for two or three minutes, the pieces were taken out with forceps, rolled upon

clean filter paper, and placed in the glass dishes previously prepared.

Examination to determine results was made by cutting sections of the cylindrical specimens, always about 5 mm. from the end. The sections were mounted in water and examined immediately after mounting.

PART I

Before making any experiments with zinc sulphate, several kinds of tissue were tested for their power to produce callus and meristem under the conditions to be used for the control. It was found that *Allium Cepa* (onion) could not be used because it decayed in the moist atmosphere. *Radicula Armoracia* (horse-radish) formed a suberized layer with meristematic tissue inside it, but was so susceptible to moulds that its use was abandoned. During the course of the experiments, *Brassica Rapa* (turnip) was tried, but it, too, proved unsatisfactory, because it was too easily affected by moulds and rot. *Helianthus tuberosus* (Jerusalem artichoke) was experimented with at the same time, but did not give the desired response. *Daucus Carota* (carrot) formed callus but so very slowly that it was considered unfavorable for experimentation. After this process of elimination, those which at first appeared to lend themselves favorably to the work in hand were *Beta vulgaris* (beet), *Ipomœa Batatas* (sweet potato), *Brassica oleracea* (kohl-rabi), *Solanum tuberosum* (potato), and, later, *Tragopogon porrifolius* (salsify). Of these the potato was quite the most satisfactory.

The sweet potato, which formed callus very readily at first, proved to be very suspectible to rot. It was probably due partially to this that the results obtained were so variable that they could not be used as a basis for any conclusions.

The beet, also, which seemed very desirable in the preliminary tests, did not form callus so readily after the experiments had been in progress for a few weeks, but dried out so much as to be useless for examination. When this trouble was encountered, freshly grown Bermuda beets were secured, but they gave no better satisfaction than the old ones. The results that were obtained, as in the case of the sweet potato, were so variable that no conclusions

could be drawn with regard to them. The variability in the results, in this instance, was largely due to the presence of vascular bundles running through the pieces used for experimentation.

The presence of the vascular bundles also confused, somewhat, the results obtained from salsify. In four cases out of seven, however, when the control pieces and the poisoned pieces were cut as

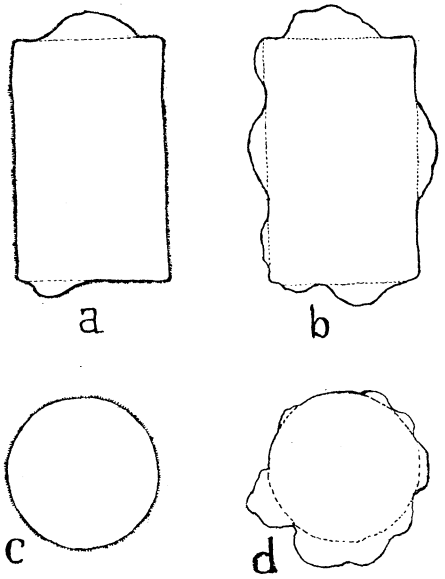

FIGURE 2. Transverse and longitudinal sections of cylinders of tissue from "kchl-rabi" for comparison of extent of callus formation with and without stimulation by zinc sulphate. *a* and *c* are the control specimens; *b* and *d* were stimulated with N/12 zinc sulphate. The drawings represent the condition two weeks after cutting, the stimulated specimens having been treated at once after the cylinders of tissue had been removed. Magnified 3 diam.

nearly alike as it was possible to make them, and always taken from the same plant, the specimens dipped in twelfth- and fourteenth-normal zinc sulphate solutions showed a greater formation of callus than the control specimens. In the other three experiments, the stimulated specimens formed less callus than the control specimens. In one of these three experiments, however, more meristem had formed in the poisoned piece than in the control.

In the experiments with the kohl-rabi, the same precautions were taken as with salsify, because of the presence of bundles.

Nevertheless, this was one factor which made the callus formation irregular, although it does not fully account for the fact that in most instances the callus formed in clumps, instead of making a uniform covering over the entire surface of the specimen. For this reason the observations on the gross appearance were thought to be of greater significance than any microscopical observations made on the specimens would be. In regard to the formation of meristem beneath the surface, none at all was formed in many experiments, even when callus was abundantly present. Its formation seemed to be dependent somewhat upon the age of the plant, and was too variable to furnish a basis for any conclusion. In regard

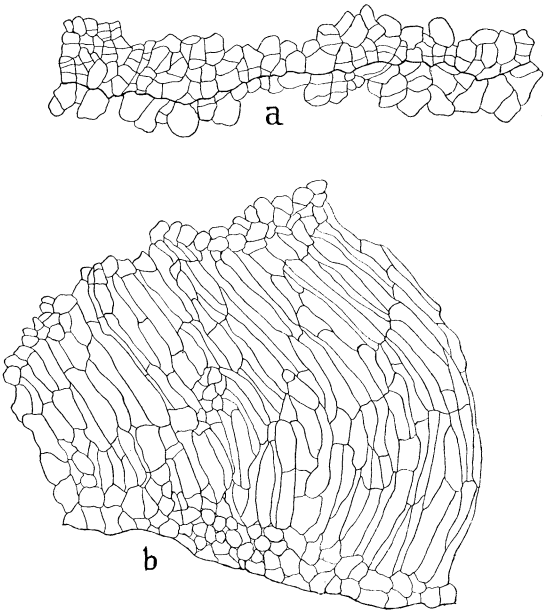

FIGURE 3. Sections of stimulated and unstimulated tissue of "kohl-rabi," cut vertically to the surface. Two weeks after setting up experiment; *a*, showing the callus formation as it appears pretty evenly all over the surface of the tissue, in the control; *b*, showing the callus formation in tissue stimulated with N/12 zinc sulphate, which is more irregular in its formation. See FIGURE 2. Magnified 38 diam.

to the callus, observations were taken upon twelve experiments using solutions of zinc sulphate from tenth to twentieth normal. In six of the experiments, the specimens poisoned with twelfth- and fourteenth-normal solutions formed callus more quickly than the

control, but irregularly and in large masses (see FIGURE 3). These pieces were also more susceptible to attacks of bacteria and moulds than were the control. In the control pieces callus was formed more slowly, but in a uniform layer over the entire surface of the specimen (see FIGURE 2). In two cases, the control had more callus formed than the poisoned pieces. In the other four experiments the results varied on the different occasions when the specimens were examined, making them valueless. Solutions stronger than tenth or twelfth normal inhibited the formation of callus. Those weaker than fourteenth normal, seemed to have little, if any, effect upon the callus formation.

Since the results in the formation of meristem in the potato were, by far, the most constant and satisfactory, the experiments on the potato will be described in detail:

EXPERIMENT I. Normal and half-normal solutions of zinc sulphate were used.

After four days:

Control. — One row of meristematic cells formed beneath the dried exterior.

N. — No meristem.

N/2. — No meristem.

After seven days:

Control. — A well-defined layer of meristem.

N. — No meristem.

N/2. — A few scattered meristematic cells two or three rows of cells beneath the surface, which was deeper than the location of the meristem in the control.

After fifteen days:

Control. — A meristematic layer several cells in thickness.

N. — Looked as if it may have recovered from the poison about two days before, as there were a great many scattered meristematic cells.

N/2. — A distinct layer of meristem a little beneath the surface.

EXPERIMENT II. Fourth- and eighth-normal solutions of zinc sulphate were used in this experiment.

After four days:

Control. — A distinct meristematic layer forming.

N/4. — No meristem.

N/8. — A number of meristematic cells but not so many as in the control.

After ten days:

Control. — A great deal of meristem close under the dried exterior.

N/4. — A good deal of meristem but not so much or so close to the exterior as in the control.

N/8. — Nearly as much as in the control. Also close to the exterior.

After twenty-one days:

Control. — A very thick layer of meristem.

N/4. — A great deal of meristem but not so much as in the control.

N/8. — Layer of meristem varies in thickness. Sometimes about the same as in the specimen poisoned with N/4 $ZnSO_4$ and sometimes nearly as thick as in the control.

EXPERIMENT III. Tenth- and twelfth-normal solutions of zinc sulphate were used.

After six days:

Control. — One row of cells meristematic.

N/10. — Almost the same as in the control.

N/12. — More meristem than in the control.

After fourteen days:

Control. — Outer meristematic cells dried and new layer forming within.

N/10. — About the same as in the control.

N/12. — Much heavier meristematic layer than in the control. (See FIGURE 4.)

EXPERIMENT IV. Twelfth- and fourteenth-normal solutions were used.

After five days:

Control. — Fairly thick layer of meristem.

N/12. — A little more meristem than in the control.

N/14. — More meristem than in the control — about the same as in the specimen stimulated with N/12 $ZnSO_4$.

After twelve days:

Control. — Heavy layer of meristem.

N/12. — About the same as in the control — impossible to say definitely whether there is more or less meristem formed.

N/14. — About the same as in the other two specimens.

After twenty-one days:

Control. — Exposed tissue dried to the edge of the meristematic layer, and some new meristem forming inside the first layer.

N/12. — Quite a little more meristem than in the control.

N/14. — About the same amount as in the specimens poisoned with N/12 $ZnSO_4$.

In view of these results it seems fair to make the following conclusions. Twelfth- and fourteenth-normal solutions of zinc sulphate stimulate the formation of meristematic tissue in the potato. A tenth-normal solution neither stimulates nor inhibits the forma-

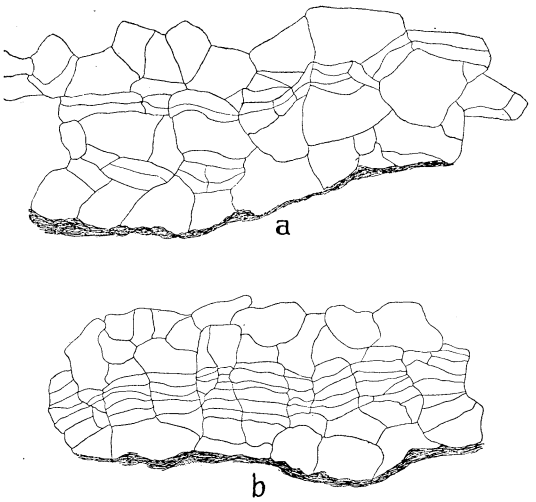

FIGURE 4. Section of stimulated and unstimulated tissue of potato tuber, cut vertically to surface. Three weeks after setting up experiment; *a*, showing the callus formation in control specimen; *b*, showing callus formation in tissue subjected to the stimulus of N/12 zinc sulphate. Magnified 53 diam.

tion of meristem. The tissue recovers from the effects of eighth-normal solution in about three or four days, from fourth-normal solution in eight or ten days, from half-normal solution in about seven days, and from the normal solution in from thirteen to fifteen days. Owing to the method by which the solutions must necessarily be applied, it is, of course, impossible to determine definitely

the exact concentration of zinc sulphate which reaches the cell. In this method, however, the critical or optimum point of concentration for this tissue is the twelfth and fourteenth normal.

PART II

In the second part of the work, the object was to determine the effect of the zinc sulphate stimulation upon the respiration of the storage tissue. Since the results with the potato in the foregoing experiments were the most nearly constant, it was the only tissue used in these latter experiments. The pieces were prepared in exactly the same manner as those used in Part I. They were then put into flasks which had been previously prepared in the following manner: The flask was weighed, then filled with distilled water, and weighed again. The first weight was subtracted from the second, the remainder being the number of grams of water the flask contained, or the volume of the flask in cubic centimeters (1 gm. H_2O = 1 c.c.). The three flasks used were always labeled I., II., and III. The volume of flask I. was 94.093 c.c.; the volume of flask II., 90.506 c.c., and that of flask III., 105.433 c.c. Flask I. was always used for the control. Before using, the flasks were always thoroughly washed in tap water, then rinsed three times in distilled water, and allowed to dry over night in an inverted position. Whenever it was necessary to use them again on the same day they were washed, they were dried in a drying oven at 100° C.

In the first experiment ten pieces of potato were used; in the second, fifteen; and in all the following experiments, twelve. After the pieces had been dipped and rolled on filter paper, they were put into the respective flasks, and cotton stoppers were inserted in the mouths of the flasks, which were then inverted over mercury cups (see FIGURE 1, *b*).

For analyzing the gas the Bonnier-Mangin instrument for gas analysis was used. In transferring the flasks from the mercury cup to the Bonnier-Mangin apparatus, a moistened finger tip was always held over the mouth of the flask until it was inserted in the mercury cup of the gas-analyzer. Before inserting it in the cup, the pieces in the flask were always shaken about a little to make sure that the gas in the flask would be uniform in composition.

The gas in every flask was analyzed twice and the average of the two results taken. Between each analysis hydrochloric acid was run in to remove all traces of the potassium hydroxide, after which the apparatus was rinsed three times with distilled water.

STIMULATED				CONTROL		DIFF. IN C C. OF CO_2 IN CONTROL AND STIMULATED. + = control more — = control less
Strength of $ZnSO_4$ sol.	Original per cent. of CO_2	Per cent. of CO_2 corrected for vol. of flask	Actual amount of CO_2 in c.c.	Original per cent. of CO_2	Actual amount of CO_2 in c.c.	
$\frac{N}{2}$	12.01	11.46	9.46	16.93	15.93	+ 6.47
	7.61	7.27	6.29	12.63	11.89	+ 5.60
$\frac{N}{12}$	12.40	11.84	10.05	13.48	12.68	+ 2.63
	10.41	9.94	8.44	10.67	10.14	+ 1.70
	8.59	8.21	6.97	9.52	8.96	+ 1.99
$\frac{N}{14}$	16.35	15.64	13.25	16.67	15.69	+ 2.44
	16.90	19.15	16.26	16.67	15.69	— 0.57
$\frac{N}{16}$	17.87	20.26	17.19	15.44	14.53	— 2.66
	17.80	20.18	17.13	17.54	16.50	— 0.63
	16.90	19.18	16.27	16.11	15.16	— 1.11
$\frac{N}{24}$	16.12	15.43	13.07	16.11	15.16	+ 2.09
	18.50	17.67	15.00	21.03	19.79	+ 4.79
$\frac{N}{32}$	18.14	17.33	14.71	17.54	16.50	+ 1.79
	20.96	23.76	20.16	21.03	19.79	— 0.37

It may be of interest to describe the method of analysis and the method of calculating results. A sample of gas was drawn into the tube of the apparatus and measured. It measured, for example, 51.10 c.mm., the correction having been made for the water present in the tube. Then a ten per cent. solution of potassium hydroxide was drawn in, run back and forth in the tube several times, and the remaining gas measured. In this case it was 43.05 c.mm., the difference between the two amounts, 8.05 c.mm., or 15.75 per cent., being the amount of carbon dioxide present in the sample. In the next analysis, 16.5 per cent. was the result gotten, the average being 16.125 per cent. This experiment was performed in flask II., the volume of which was 90.306 c.c. But 9.236 c.c. of the flask was occupied by the twelve pieces of potato 20 mm. long and 7 mm. in diameter. Therefore the complete amount of gas present in the flask was 81.07 c.c., 16.125 per cent. of which, or 13.072 c.c., was carbon dioxide. The volume of the

control flask was 94.093 c.c. Subtracting the bulk of potato, the volume of gas present would be 84.857 c.c. If 13.072 c.c. of the total gas volume, 84.857 c.c., was carbon dioxide, the per cent. of carbon dioxide present would be 15.43. This is an example of the method used in all the analyses and calculations, the results of which will be found on page 499.

These investigations are merely the introduction to what might be done in the way of experiments showing the effect of zinc sulphate stimulations upon the respiration of storage tissues, but they serve to indicate that fourteenth-normal solutions, or any solutions stronger than that, inhibit the respiration of the storage tissue of potato. A sixteenth-normal solution stimulates respiration. Further than that, the results so far obtained do not justify any definite statements. It is hoped that at some future time the data already obtained in these investigations may be amplified and the work carried on to a far greater extent.

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